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Full Length Article

A contribution to the specification of Caesalpinioideae (L) based on morphological and molecular criteria

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ARTICLE INFO

Article history:
Received 10 January 2013
Accepted 15 March 2013
Available online 1 November 2013

Keywords:
Morphology
Leaf architecture
Stomatography
Biochemical markers
Caesalpinioideae

ABSTRACT

The present study included the investigation of both morphological attributes of some taxa of Caesalpinioideae *viz.* whole plant, leaf architecture & epidermal characteristics (LM & SEM) and certain molecular attributes (RAPD & Isozymes) to clarify the diversity and the diagnostic importance of these characters. The sum of both character states of morphological and molecular criteria (326 attributes & 353 bands) respectively of total (679 attributes) of the investigated taxa were subjected to a numerical analysis using NTsys-pc program (version 2.02). The resulted dendrogram interpreted the similarities and dissimilarities between the investigated taxa. The specific relationships were discussed and compared with some of the current systems of classification. The aim of the present study is tried to find the interspecific relationships of the studied taxa through the investigation of their morphological and molecular characters in addition to a numerical evaluation of such characters. Among the reached concluding remarks, The dendrogram resulted from morphological and molecular attributes supported the separation of *Cassia* and *Senna* as two taxonomic entities.

1. Introduction

Caesalpinioideae includes 171 genera and about 2250 species of tropical and sub-tropical trees and shrubs (Lewis et al., 2005). Boulos (1999) recorded the following wild species in Egyptian flora *viz.* *Cassia italica*, *Cassia holosericea*, *Cassia occidentalis*, *Cassia senna* and *Delonix elata*.

The principal characteristics of the leaf venation pattern of a species are genetically fixed. This provides the basis for using the leaf venation as a taxonomic tool (Hickey, 1973; Roth-Nebelsick et al., 2001). Seetharam and Kotresha (1998) emphasized the taxonomic importance of venation and its usefulness in classification of *Bauhinia* L.

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Peer review under the responsibility of Beni-Suef University

Production and hosting by Elsevier

2314-8535/$ – see front matter
http://dx.doi.org/10.1016/j.bjbas.2013.03.004

Leaf epidermal studies have shown that stomata can provide valuable taxonomic and systematic evidence in both living and fossil plants and also have played a significant role in framing hypotheses about early angiosperm evolution (Carpenter, 2005). Zou et al. (2008) examined the leaf epidermal micro characters of nine taxa of *Cercis* using SEM & LM, and then concluded that the interspecific differences are minor in the genus.

RAPD markers are powerful techniques for determining intra- and interspecific genetic variations and allow direct comparison of plant variation at both biochemical and molecular levels (Williams et al., 1990; Welsh and McClelland, 1990; Carlier et al., 2004). RAPD markers have been reported to be as efficient as AFLP, SSR, RFLP and ISSR markers (Martins et al., 2003; Zahuang et al., 2004) for genetic analysis at different plant species.

Whitty et al. (1994) adopted RAPD method for use as a phenetic tool on the legume tribe Cassiinae, using eight primers and showed the potential for separation of the nodulated nitrogen fixing genus *Chamaecrista* from the previously congeneric groups *Cassia* and *Senna*. Diallo et al. (2007) studied 10 *Tamarindus* populations using markers RAPDs, the results showed that *Tamarindus indica* has a high intra population genetic variability.

Despite the use of DNA markers, isozymes are still widely employed in species delimitation, conservation and cultivar identification (Samec et al., 1998; Mohamed, 2006). Isozymes have been applied as molecular-genetic markers to study genetic diversity and phylogenetic affinities in populations of *Gleditsia triacanthos* (Schnabel and Hamrick, 1990), Cassia species (Nualkaew et al., 1998; Siva and Krishnamurthy, 2005).

Concerning numerical analysis, several authors checked the current classification for different genera and species of Leguminosae and analysed their results by using different numerical analysis programs. Larmarque and Fortunato (2003) used the numerical analysis to discuss the taxonomic placement of *Acacia emiliona* and its affinity within subgenus *Aculeiferum*. Tantawy et al. (2005) showed the similarities between some of different taxa of Mimosoideae. El-Gazzar et al. (2008) reached to computer-generated keys to the flora of Egypt (Mimosoideae & Caesalpinioideae). Abou El-Enain et al. (2007) delimited the genus *Cassia* into two subgenera *viz. Fistula* and *Senna* based on the basis of morphological criteria and seed protein electrophoresis.

The aim of the present study is tried to find the interspecific relationships of the studied taxa through the investigation of their morphological and molecular characters in addition to a numerical evaluation of such characters.

2. Materials and methods

Fresh mature leaf materials of 25 caesalpinioid taxa grown in some Egyptian botanical gardens were collected and subjected for the present study (Table 1). Identification was confirmed according to (Bailey, 1949; Bircher, 1960).

The taxa were further matched against dried specimens in the Herbaria of Ain Shams University (CAIA), Cairo University (CAI), Flora & Phytotaxonomy & Agriculture Research Center (CAIM) and Orman Botanical Garden. Voucher specimens of the studied taxa are deposited in CAIA.

Table 1 – Collection data of Caesalpinioideae.

No.	Taxa	Source
01	*Bauhinia alba* Buch.-Ham. ex Wall.	OBG
02	*B. hookeri* F. Muell.	BGC
03	*B. variegata* L.	BGA
04	*Brownea grandiceps* Jacq.	ZBG
05	*Caesalpinia ferrea* Tulasne.	ZBG
06	*C. gilliesii* (Wallich ex Hook.) Dietr.	BGC
07	*Cassia fistula* L.	OBG
08	*C. grandis* L.f.	ZBG
09	*C. javanica* L.	ZBG
10	*C. marginata* Roxb.	OBG
11	*C. nodosa* Buch-Ham. ex Roxb.	OBG
12	*Ceratonia siliqua* L.	BGA
13	*Cercis chinensis* Bunge	OBG
14	*Delonix regia* (Bojer ex Hook.) Raf.	BGA
15	*Gleditsia caspica* Desf.	OBG
16	*Haematoxylum campechianum* L.	BGA
17	*Parkinsonia aculeata* L.	BGA
18	*Peltophorum africanum* Sond.	BGA
19	*Saraca indica* L.	OBG
20	*Schotia brachypetala* Sond.	OBG
21	*Senna alata* (L.) Roxb.	BGC
22	*S. didymobotrya* (Fres.) Irwin & Barneby	BGA
23	*S. sophera* (L.) Roxb.	ZBG
24	*S. surattensis* (Burm. f.) Irwin & Barneby	ZBG
25	*Tamarindus indica* L.	OBG

BGA: Botanical Garden, Ain Shams University, Faculty of Science, Cairo, Egypt. **BGC:** Botanical Garden, Cairo University, Faculty of Agriculture, Giza, Egypt. **OBG:** Orman Botanical Garden, Ministry of Agriculture, Giza, Egypt. **ZBG:** Zohria Botanical Garden, Ministry of Agriculture, Gezzera, Cairo, Egypt.

Macromorphological attributes of the whole plant were described from the investigated specimens or compiled from text books *viz.* Bailey (1949).

Lamina vein architecture was carried out according to the customary method of Jesudass et al. (2003). Leaf architectural terminology generally follows Hickey (1973) and LAWG (1999).

Stomatography was carried on the bases of traditional method of Stace (1965). The photomicrographs were taken using a Reichert Microstar IV microscope at the Plant Taxonomy Research Laboratory, Botany Department, Faculty of Science, Ain Shams University, Cairo, Egypt. For SEM small pieces (7 mm^2) of the leaf material were fixed on SEM stubs with double-sided tape, coated with gold in SPI-Module sputter coater, examined and photographed in Jeol JSM 5200 at different magnifications ranged from 750 X-1500X. Descriptive

Table 2 – Primers used in RAPD analysis.

No.	Primer	Sequence
1	SC10-5	TCGGAGTGGC
2	SC10-14	TCCCGACCTC
3	SC10-17	GTTAGCGGCG
4	SC10-18	GCCCTACGCG
5	SC10-22	CTAGGCGTCG
6	SC10-23	GGCTCGTACC
7	SC10-25	CGGAGAGTAC
8	SC10-59	GCATGGAGCT
9	SC10-64	CCAGGCGCAA
10	SC11-30	CCGAAGCCCT

terminology of epidermal characteristics based on Metcalfe and Chalk (1950), Murley (1951), LAWG (1999) and Prabhakar (2004).

Genomic DNA extraction was performed as suggested by DNA extraction kit's manufacturer Jena Biosciences, Plant DNA Preparation Kit. Polymerase chain reactions (PCR) were carried out according to Whitty et al. (1994) and the primers used are presented in Table 2.

The utilized isozymes are α- and β-esterase (α- and β- Est), acid phosphatase (Acph), alcohol dehydrogenase (Adh), and aldehyde oxidase (Alo). These isozymes were separated in 10%

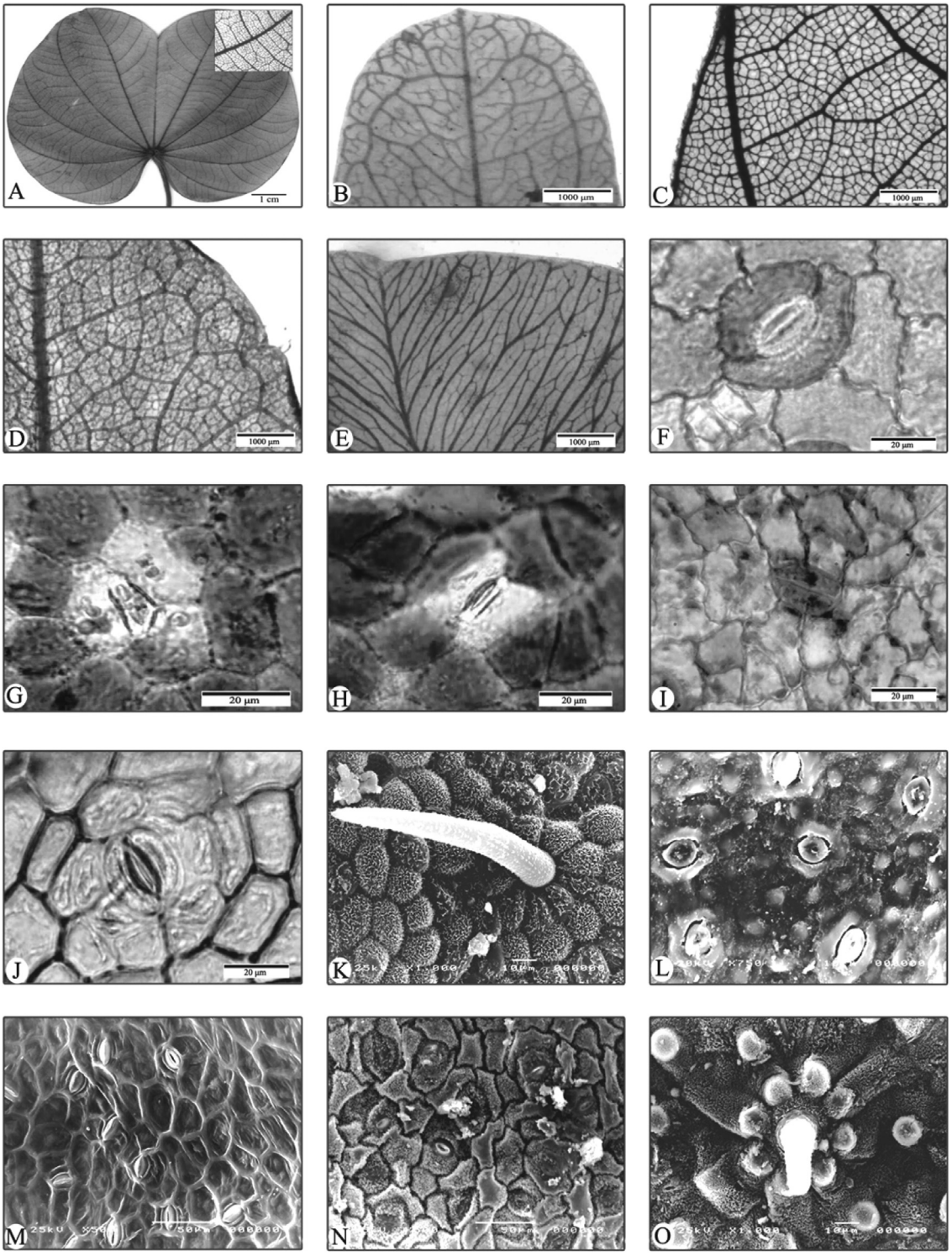

Fig. 1 – A&B, Major primary vein categories of lamina architecture; A, Campylodromous. B, Pinnate. C-E, Major secondary vein categories of lamina architecture; C, Brochidodromous. D, Festooned brochidodromous. E, Cladodromous. F-J, Major types of stomata; F, Paracytic. G, Isotricytic. H, Tetracytic. I, Anomocytic. J, Cyclocytic. K–O, Major types of lamina surface sculpture (SEM); K, Colliculate. L, Pusticulate. M, Reticulate. N, Ruminate. O, Tuberculate.

polyacrylamide gel electrophoresis according to Stegemann et al. (1985). Isozymes extraction and electrophoresis were carried out on the bases of traditional method. In gels staining protocols of Wendel and Weeden (1989) were used for Acph, Jonathan and Wendel (1990) were used for Adh & Alo and Scandalios (1964) were used for α- and β- Est. Gels were washed two or three times with tap water, fixed in EtOH/20% glacial acetic acid; 9:11 v/v. for 24 h and then was photographed.

Unweighted Pair-Group Method using Arithmetic Averages with SAHN function (Sneath and Sokal, 1973) was used to estimate states of characters variation among the species, each taxa was considered as operational taxonomic unit (OTU) and states of characters analysed as binary characteristics. The formation of groups is depending on the values of similarity. All computations were carried out by the aid of the NTSYS-PC version 2.02 (Rohlf, 2000).

3. Results

3.1. Morphologicl traits

Leaf composition is a valuable taxonomic value led to the separation of *Bauhinia* and *Cercis* (simple leaf) from the remaining studied taxa this is comparable to the current taxonomic systems of classification, to cite but a few one can refer to Bentham and Hooker (1862), Engler (1964), Willis (1966), Hutchinson (1967), Pettigrew and Watson (1977), Smith (1977), Pollhill and Raven (1983), Watson and Dallwitz (1983) and Lewis et al. (2005), where *Bauhinia* and *Cercis* grouped in tribe Bauhinieae or Cercideae. Pettigrew and Watson (1977) segregated *Haematoxylum* away from the rest of related taxa *viz*. *Ceasalpinia*, *Delonix*, *Parkinsonia* and *Peltophorum*. The data in the present study supported the segregation of *Haematoxylum* and *Gleditsia* (oncepinnate & bipinnate, paripinnate) away from the related taxa. In the current study the shape of the blade distinguished *Bauhinia* sp. under investigation away from *Cercis chinencis*. In the former the blade was suborbicular and cordate in the latter. This conclusion is comparable with the work of Wunderlin et al. (1981, 1987) "tribe Cercideae or Bauhinieae is divided into two subtribes *viz*. **Cercidinae** (*Cercis*) and **Bauhiniinae** (*Bauhinia*)". The data in the present study about the union of sepals supported the conclusion reached before by Rendle (1925) "Bauhinieae have a gamosepalous calyx" except *Bauhinia hookeri* (polysepalous one). The filament form (sigmoid & noduated) in taxa under investigation was comparable to those mentioned by Bentham (1871), Taubert (1891) and Randell (1976) who suggested that the filament form help in segregation of genus *Cassia* L. into three subgenera *viz*. *Fistula*, *Senna* and *Lasiorhegma*, or subgenera *viz*. *Cassia*, *Senna* and *Absus* respectively. Rendle (1925) distinguished tribe Cassieae by having more or less basifixed anthers. This conclusion is in agreement with the data extracted in the present study. Bentham (1871), Taubert (1891) and Randell (1976) concluded that the flattened and terete ovary enhance the separation of *Cassia* and *Senna*. This conclusion is in agreement with the data extracted in the present study. The campylodromous type of primary vein category (Fig. 1) was considered unique character for *Bauhinia* & *Cercis*. This is in accordance with the conclusion reached before by many systems of classification, where the studied *Bauhinia* sp. and *C. chinensis* were grouped under the same tribe Bauhinieae or Cercideae. The festooned brochidodromous type of secondary vein category (Fig. 1) segregated *C. chinensis* away from the studied *Bauhinia* sp. and supported the division of tribe Cercideae or Bauhinieae into two subtribes, **Cercidinae** and **Bauhiniinae** by Wunderlin et al. (1981, 1987). The studied *Cassia* species (hypostomatic) were separated from *Senna* species (amphistomatic) in the present study, this is in accordance with Bentham (1871), Taubert (1891) and Randell (1976) who segregated genus *Cassia* into three subgenera *viz*. *Fistula*, *Senna* and *Lasiorhegma*.

3.2. Molecular differentiation

The high discriminatory power of the primers used indicated that the RAPD technique provides an effective tool for delimitation in Caesalpinioideae.

All primers produced 323 bands and showed no monomorphic bands (Fig. 2), meaning that the polymorphism investigated by these primers reached 100%.

The amplifications products of the **primer SC10-5** illustrated that two unique bands were scored in *B. hookeri* at about 11.764 bp and 7.680 bp. One unique band was scored at about 2.015 bp in *Delonix regia*. **Primer SC10-14** Showed eight unique bands helped in the separation of five taxa, *viz*. 1.646 bp for *Bauhinia alba*, about 95.516 bp, 41.137 bp and 5.934 bp for *B. hookeri*, about 12.304 bp and 2.637 bp for *Cassia grandis* and *Cassia marginata* respectively and about 108.312 bp and 1.172 bp for *Gleditsia caspica*. Seven unique were recognized by **primer SC10-17** that identify the following taxa *viz*. *Bauhinia variegata* at about 43.693 bp and 30.949 bp, *Brownea grandiceps* at about 72.897 bp and 1.367 bp, *Cassia fistula*, *Cassia nodosa* and *Peltophorum africanum* at 19.057 bp, 2.986 bp and 46.863 bp respectively. **primer SC10-18** produced ten unique bands for the following taxa *viz*. *B. variegata* (at about 76.264 bp), *B. grandiceps* (1.923 bp), *Caesalpinia ferrea* (1.785 bp), *Cassia javanica* (68.453 bp), *C. marginata* (3.278 bp), *D. regia* (5.224 bp and 1.300) and *Haematoxylum campecianum*, *Saraca indica* & *Senna surattensis* (3.778 bp, 5.051 bp and 19.626 bp) respectively. **Primer SC10-22** produced 12 unique bands distinguished the following taxa *viz*. *B. hookeri* at about 0.385 bp, *B. variegata* at 7.097 bp, *Caesalpinia gilliesii* at about 11.570 bp, *C. fistula* at about 12.987 bp & 5.759 bp, *C. javanica* at about 13.883 bp, *C. nodosa* at 6.047 bp, 3.709 bp & 2.879 bp, *Ceratonia siliqua* at about 5.759 bp & 0.948 bp and *T. indica* at 2.754 bp. **Primer SC10-23** generated nine unique bands that identified the following taxa *viz*. *B. alba* at about 22.779 bp, *C. nodosa* at about 4.125 bp and 1.862 bp, *C. siliqua* at about 0.587 bp, *C. chinensis* at about 29.696 bp, 24.521 bp and 9.551 and *P. africanum* & *S. indica* at about 11.343 bp and 3.012 bp respectively. Eleven unique bands were recognized by **primer SC10-25** for *B. hookeri* (0.953 bp), *C. gilliesii* (5.912 bp), *C. fistula* (27.005 bp and 0.848 bp), *C. marginata*, *C. nodosa* and *C. siliqua* (11.336 bp, 4.257 bp & 3.705 bp) respectively, *D. regia* (21.377 bp and 5.623 bp) and *G. caspica* & *P. africanum* (0.771 bp and 8.583 bp) respectively. Scorable 13 unique bands using **primer SC10-59** were recognized in 12 taxa at about molecular weight 2.116 bp, 3.553 bp, 3.257 bp, 4.416 bp, 1.102 bp, 1.241 bp, 2.858 bp and 0.585 bp in *B.*

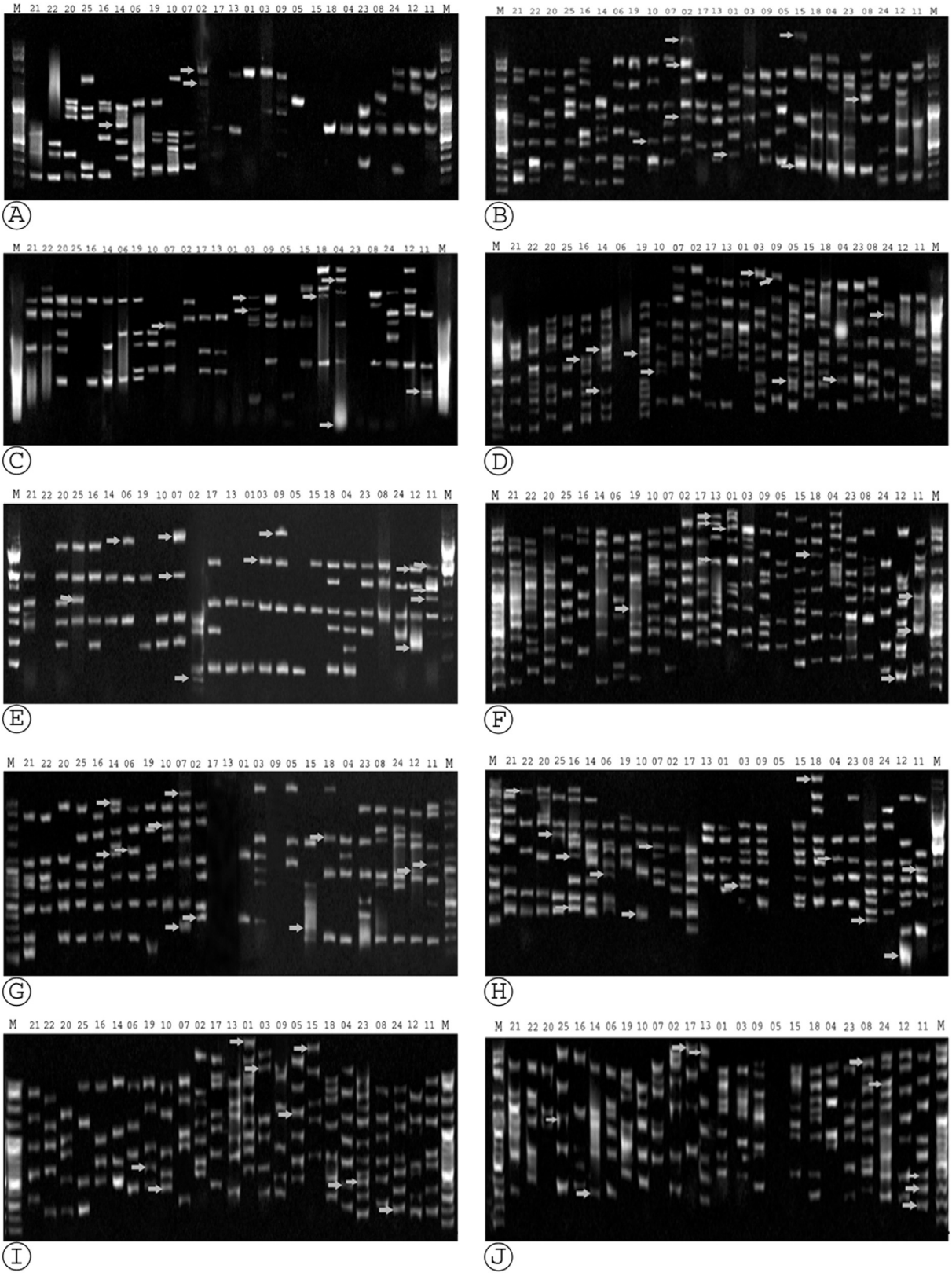

Fig. 2 – A-J, RAPD profile of the studied taxa of Caesalpinioideae generated by A, primer SC10-5. B, primer SC10-14. C, primer SC10-17. D, primer SC10-18. E, primer SC10-22. F, primer SC10-23. G, primer SC10-25. H, primer SC10-59. I, primer SC10-64. J, primer SC11-30.

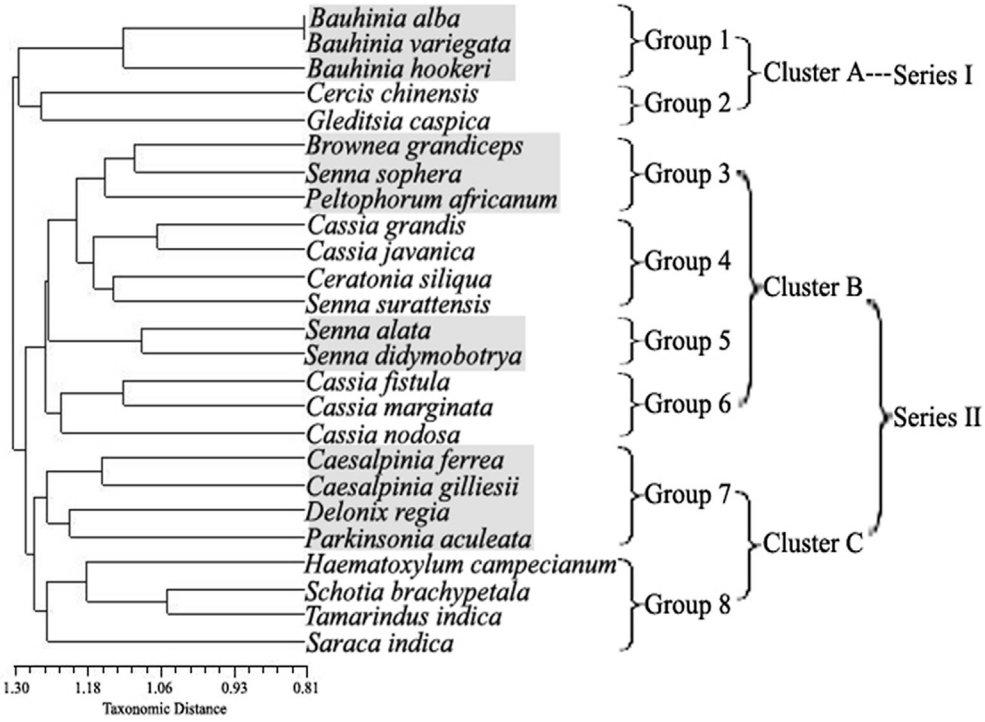

Fig. 3 – Dendrogram based on morphological and molecular characters of the studied taxa of Caesalpinioideae using NTSYS – pc program version 2.02.

variegata, *B. grandiceps*, *C. gilliesii*, *C. fistula*, *C. grandis*, *C. marginata*, *C. nodosa* & *C. siliqua* respectively. At about 3.653 bp and 1.414 bp in *H. campecianum*. At about 15.718 bp, 12.300 bp and 5.555 bp in *P. africanum*, *Senna didymobotrya* & *T. indica* respectively. **Primer SC10-64** produced nine unique bands that recognized for the identification of the following taxa *B. alba* (65.382 bp), *B. variegata* (36.504 bp), *B. grandiceps* (1.637 bp), *C. ferrea* (11.064 bp), *C. marginata* (1.522 bp), *G. caspica* (60.788 bp), *S. indica* (2.492 bp), *Senna sophera* (1.790 bp) and *S. surattensis* (0.850 bp). The profile of **primer SC11-30** showed that nine unique bands identified the following studied taxa *viz. C. grandis* (15.850 bp), *C. nodosa* (1.678 bp, 1.356 bp and 0.969 bp), *C. chinensis* (18.745 bp), *D. regia* (1.247 bp), *Parkinsonia aculeata* (20.000 bp), *S. surattensis* (10.381 bp) and *T. indica* (5.389 bp).

The highest number of bands (three) produced by **Acid phosphatase** isozyme system was found only in *C. ferrea*, which gave maximum gene/gene expression of acid phosphatase isozyme. The lowest number of acid phosphatase bands (one each) was found in *B. alba*, *B. grandis*, *C. gilliesii*, *C. grandis*, *C. javanica*, *C. marginata*, *P. aculeata*, *S. indica*, *Schotia brachypetala* and *T. indica*, which gave minimum gene/gene expression of the same isozyme. Four species-specific bands were detected in *C. fistula*, *H. campecianum*, *S. indica* and *S. didymobotrya*, the remaining three bands were shared by at least two taxa. No unique bands detected among the four polymorphic ones generated by **Alcohol dehydrogenase** isozyme system. The highest number of bands (three) was found only in *C. ferrea*. The lowest number of alcohol dehydrogenase bands (one each) was found in *B. hookeri*, *B. variegata*, *B. grandiceps*, *C. fistula*, *C. grandis*, *C. marginata*, *C. nodosa*, *C. siliqua*, *H. campecianum*, *S. sophera* and *T. indica*. The zymogram of **α-esterase** revealed that ten bands were detected in 20 taxa, ranging from one to eight bands per taxa. The highest number of bands (eight) was found only in *H. campecianum*, which gave maximum gene/gene expression of α-esterase isozyme. The lowest number of α-esterase bands (one each) was found in *B. alba*, *B. hookeri*, *C. ferrea*, *C. grandis*, *C. nodosa*, *S. brachypetala*, *S. sophera*, *S. surattensis* and *T. indica*. Three species-specific bands were detected in *H. campecianum*, the remaining seven bands were shared by at least two taxa. A total of five polymorphic bands were generated by **β-esterase** isozyme system that detected in nine taxa, ranging from one to three per taxa. The highest number of bands (three) was found only in *Senna alata*. The lowest number of β-esterase bands (one each) was found in *C. gilliesii*, *C. fistula*, *P. aculeata*, *S. didymobotrya*, *S. sophera* and *T. indica*, which gave minimum gene/gene expression of the isozyme.

One species-specific band was detected in *C. ferrea* and another one in *S. alata*, the remaining three bands were shared by at least two taxa. Out of four polymorphic bands produced by **Aldehyde oxidase** isozyme system in 18 taxa, only one unique band observed in *S. alata*. The highest number of bands (three each) was found in *S. alata* and *S. didymobotrya*, which gave maximum gene/gene expression of aldehyde oxidase isozyme. The lowest number of aldehyde oxidase bands (one each) was found in *B. hookeri*, *B. variegata*, *C. ferrea*, *C. marginata*, *C. nodosa*, *C. siliqua*, *C. chinensis*, *D. regia*, *G. caspica*, *H. campecianum*, *P. africanum*, *S. surattensis* and *T. indica*. All the enzyme systems analyzed were polymorphic where the interspecific polymorphism reached 100%.

The data extracted from RAPD-PCR for the studied taxa were amalgmated with the data from morphological and

isozyme analyses then subjected to numerical analysis to interprete and discuss the interrelationship between the taxa under investigation at generic and specific level, also to compare the schematic presentation with some of current systems of classification. The taxonomic treatment based on 679 attributes (326 morphological attributes and 353 molecular attributes) used for computation and produced dendrogram revealed classification of the studied taxa of Caesalpinioideae which compared with the current system treatments. The resulted dendrogram showed that the taxa under investigation were split into two series, three clusters and eight groups (Fig. 3).

4. Discussion

The generated dendrogram clarifies that the taxa under investigation divided into two main series (I and II) at taxonomic distance 1.3. Series I includes one cluster (A) with two groups (1 and 2). Cluster A with group 1 and 2 includes five studied taxa. Series II includes two clusters (B andC), cluster B with four groups (from 3 to 6) including 12 studied taxa while cluster C with two groups (7 and 8) including eight taxa. The interrelationships between these taxa are summarized as follows.

Series I, Group 1: includes *B. alba*, *B. variegata* and *B. hookeri* which separated at the taxonomic distance of 1.12.

Group 2: includes *C. chinensis* & *G. caspica* which separated at taxonomic distance 1.26.

The grouping of studied *Bauhinia* sp. and *C. chinensis* in one cluster and two closely related groups (cluster A, Group 1 & 2) is comparable with current system of treatment of Caesalpinioideae where *Bauhinia* and *Cercis* classified under Tribe Bauhinieae or Cercideae. Wunderlin et al. (1981, 1987) suggested the division of tribe Cercideae or Bauhinieae into two subtribes, **Cercidinae** and **Bauhiniinae**. The data extracted from cluster A, group 1 & 2 encourage this suggestion (studied *Bauhinia* species are classified under subtribe Bauhinieae while *Cercis* under subtribe Cercineae).

According to Watson and Dallwitz (1983), *G. caspica* (tribe Caesalpinieae), separated away from *Caesalpinia*, *Delonix*, *Parkinsonia* and *Peltophorum* in a separate subgroup. *G. caspica* in the present study and on the bases of morphological and molecular criteria was separated away from tribe Caesalpinieae and grouped with *C. chinensis* (group 2 at 1.26 taxonomic value). It was suggested that data extracted enhance the grouping of this taxa with *C. chinensis* under subtribe Cercidinae.

Series II, Group 3: includes *B. grandiceps*, *S. sophera* & *P. africanum* at a taxonomic value 1.16. According to the different authors as mentioned in Tables 1and 2 in the present study, *Brownea*, *Tamarindus*, *Saraca* and *Schotia* were grouped under tribe Amhestieae. Hutchinson (1967) and Watson and Dallwitz (1983) separated *Brownea* away from the related taxa *viz. Tamarindus*, *Schotia* & *saraca* in a separate subgroup. In the present study the morphological and molecular data supported the suggestion of Hutchinson (1967) and Watson and Dallwitz (1983).

Group 4: included *C. grandis*, *C. javanica*, *C. siliqua* & *S. surattensis* separated at 1.16. The two former Cassia sp. are more closely related than Ceratonia and Senna. In this connection Irwin and Barneby (1981) divided Cassieae into five subtribes *viz.* Ceratoniinae (*Ceratonia*), Dialiinae, Duparquetiinae, Cassiinae (*Cassia*), and Labicheinae and this is in accordance with the proposed treatment in the present study.

Group 5: includes *S. alata* & *S. didymobotrya* at 1.11 taxonomic value.

Group 6: includes *C. fistula*, *C. marginata* & *C. nodosa* at taxonomic value 1.23. In the present study the morphological and molecular data supported the separation of studied *Cassia* and *Senna* species from each other and this is in agreement with Bentham (1871) and Taubert (1891) in which genus *Cassia* L. is segregated into three subgenera *viz. Fistula*, *Senna* & *Lasiorhegma* and into *Cassia*, *Senna* & *Absus* (Randell, 1976).

Group 7: includes *C. ferrea, C. gilleisii*, *D. regia* & *P. aculeata* at 1.25 taxonomic value. These taxa belong to tribe Caesalpinieae or Eucaesalpinieae in most of the current taxonomic treatments of classification.

Group 8: includes *H. campecianum*, *S. brachypetala*, *T. indica* & *S. indica* at taxonomic level 1.25. The taxa under this group represent tribe Detarieae or Amhersiteae (except *Haematoxylum*, tribe Caesalpinieae or Eucaesalpinieae) as mentioned by Bentham and Hooker (1862), Engler (1964), Willis (1966), Hutchinson (1967), Pettigrew and Watson (1977), Smith (1977), Pollhill and Raven (1983), Watson and Dallwitz (1983) and Lewis et al. (2005). *S. brachypetala* and *T. indica* are closely related at taxonomic value 1.05 and this is in contradiction with Pettigrew and Watson (1977) where *Schotia* and *Brownea* were placed together in a single subgroup, *Saraca* in another subgroup and *Tamarindus* in third one. In this respect *Haematoxylum* was delimited by Pettigrew and Watson (1977) and Watson and Dallwitz (1983), this is in accordance with the data extracted in the present study. From the proposed treatment (Fig. 1) the following subsequent points revealed a taxonomic meaning:

The majority of studied taxa are arranged under the specific tribes based on morphological and molecular attributes.

The studied taxa of Cassieae (*Cassia*, *Senna* & *Ceratonia*) are considered paraphyletic (one ancestor, *Cassia* s.l. segregated away from the remaining descendants). This is supported by Irwin and Barneby (1981), Herendeen et al. (2003) and Wojciechowski et al. (2004) who concluded that Cassieae is not monophyletic based on analysis of molecular sequence data.

The proposed treatment and dendrogram resulted from morphological and molecular attributes supported the separation of *Cassia* and *Senna* as two taxonomic entities.

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